Methods and supplemental information for Defenders' report: Biodiversity in Crisis

Literature Search: Biodiversity and Threats

We conducted a search of primary literature from a major database (Scopus) to extract the number of published scientific articles that mention biodiversity from 1991 to 2022. We based our methods off of Legagneux et al. (2018). Like Legagneux et al., we compiled scientific manuscripts from studies worldwide published in English. We used Legagneux et al.'s search terms to identify biodiversity-related papers ("biodiversity," "ecosystem services," "endangered species," "IPBES") to represent the total number of studies discussing biodiversity over this time. We then identified which papers within this group specifically discussed each of the five drivers of global biodiversity loss ("climate change," "invas*," "pollut*," "overexploit*," "overharvest*," "overfish*," "degrad*," "habitat loss," "land-use change," "sea-use change") or extinction ("Anthropocene," "biodiversity loss," "extinct*," "imperiled"). We also identified the number of papers that included terms related to both threats and biodiversity loss/extinction. We used publication year to analyze temporal trends in the number of total papers related to biodiversity, the five threats or extinction (Figure 1).

Our searches encompassed almost 300,000 papers and are representative of the total numbers and proportions of literature in the aforementioned categories. While we strove to apply search terms that would be direct and relevant to the literature represented in our findings, there will inevitably be papers included that are from inapplicable fields or that do not apply. A different set of relevant search terms would yield slightly different results. Our findings are therefore meant to depict trends rather than precise counts. All final searches were conducted February 8, 2023.

Species' Threats Determination

We used multiple sources to determine which five drivers threaten each species listed under the U.S. Endangered Species Act (ESA; n=1,662) since their time of listing: Haines et al. (2021), Delach et al. (2019), Weber et al. (2023), Wrobleski et al. (2023), International Union for the Conservation of Nature's Red List of Threatened Species (IUCN 2023), NatureServe's Explorer (NatureServe 2023), and Species Status Assessments from U.S. Fish and Wildlife Service (USFWS).

Haines et al. identified which species were threatened by six factors at the time of listing under the ESA: "habitat modification", "overutilization", "pollution", "species-species interactions", "environmental stochasticity" and "demographic stochasticity". We considered "habitat modification", "overutilization", "pollution", and "environmental stochasticity" to relate directly to "land- and sea-use change", "overexploitation", "pollution", and "climate change", respectively. "Species-species interactions" were flagged as a broader category under which the "invasive species" threat would fall. To confirm that the "species-species interactions" referenced included a non-native species, we checked additional sources. More specifically, if IUCN did not identify invasive species as a threat for a given species for which Haines identified "species-species interactions" as a threat, we searched NatureServe threat descriptions for the following terms: invas*, alien, feral, exotic, introduced, non-native, nonnative, zebra mussel, WNS (white-nose syndrome), cats. We considered invasive species a threat if any of these terms were present.

Ten species were listed under the ESA after the Haines data was published, for which we referenced the most recent USFWS assessment or listing proposal. For these species, threats were identified using the process outlined in Leu et al. (2019).

In addition to the Haines et al. analysis, we searched the IUCN threats description for each species. If the following terms were identified in the threats description and the threat was not already identified as part of the process above, we included the associated threat:

- Land and Sea-Use Change: "degrad*" "habitat loss" "land-use change" "sea-use change"
- Overexploitation: "overexploit*" "overharvest*" "overfish*"
- Invasive Species: "invas*"
- Climate Change: "climate change"
- Pollution: "pollut*"
- climate change: "climate change"

We then identified whether a threat was direct or indirect as well as hypothesized or current. Indirect threats are threats that result from one of the other threats as a primary driver (e.g., the primary driver of feral pigs causing habitat degradation is invasive species; land- and sea- use change is an indirect threat). A threat was considered hypothesized rather than current if “potential,” “may,” “could,” “expected,” and “likely” terminology was involved in the description. We incorporated all current direct threats into our analysis (Figures 5 and S1).

Lastly, if Delach et al. 2019, Weber et al. 2023 or Wrobleski et al. 2023 found a species sensitive to climate change, we included climate change as a threat.

Given this approach, threats for any species may have been added based on the literature and other published sources, but none have been removed from those identified at the time of listing. Further research may choose to adjust this more conservative approach, but will need to make explicit decisions about at what threshold a threat is no longer endangering a species. For example, some species were originally listed under the ESA due to overexploitation pressures that may have been alleviated to some extent by more recent harvest and trade regulations.

To explore the data and sources, visit our interactive dashboard: Indicator of Risks to Imperiled Species (IRIS) at https://defenders-cci.org/publication/five-drivers/.

Geospatial Data Collection and Analysis

We collected geospatial data that directly represent or serve as proxy for the five main drivers of global biodiversity loss: climate change, land-use change, invasive species, overexploitation, and pollution (see table below). All datasets were publicly and freely available, cover all the contiguous U.S. and were resampled to 1km resolution. In some cases, multiple datasets were combined to represent a single threat. Spatial correlation among datasets was tested prior to inclusion in the threat layer to avoid redundancy. To achieve this, first we log[X+1]-transformed each layer (Halpern *et al.* 2015; Di Minin *et al.* 2019) which reduced the effect of extreme outliers when rescaling the data. For integer data such as species richness and land-use change detection, pixels with true zeros were also excluded to avoid zero-inflation when rescaling. Each individual layer was rescaled between 0 and 1 and then combined by taking the mean value for each pixel (Halpern *et al.* 2015; Di Minin *et al.* 2019). For the resulting threat layer, a larger value represents higher exposure to the potential impacts of that threat. The five resulting threat layers were combined in a similar manner to create a cumulative index of threat exposure (Figure 3). All data pre-processing and analyses were done in ArcGIS Pro 3.0.3 (ESRI 2022) in USA Contiguous Albers Equal Area Conic projection.

Climate Change: We used climatic dissimilarity (Belote *et al.* 2018) and climate velocity (AdaptWest Project 2015) to describe climate change (following Dreiss *et al.* 2022). Climate velocity describes the rate of climate change and was informed by an ensemble of seven general circulation models (GCMs), which greater values representing greater exposure to climate change. Climate dissimilarity describes differences between current and future climates based on 11 bioclimatic variables. Greater values represent higher differences between current and future climate. Both datasets used future climate projections to 2080s time period based on emission scenario representative concentration pathway (RCP) 4.5.

Land-use Change: We calculated potential threat to land conversion using land cover data for 2005 and projected land cover in 2075 (pathway scenario B1; Sohl *et al.* 2014). Ten land cover categories were used: water, developed, mechanically disturbed, barren, forest, grassland, shrubland, agriculture, wetland, and ice/snow. Then we identified land cover change between 2005 and 2075, resulting in a binary 0,1 dataset where 0 represents no change and 1 represents change in land cover category between the two time-periods. We calculated the proportion of area within each 1km grid cell projected to change land classes.

Invasive Species: We collected species range data for non-indigenous birds (Dyer *et al.* 2017) and mammals (Biancolini *et al.* 2021), as well as point occurrence data for non-indigenous reptile (Wiens *et al.* 2019) and non-indigenous aquatic species (USGS 2023). We also collected habitat suitability ensemble models for 220 invasive plant species (Jarnevich *et al.* 2023). We developed a species richness dataset for each taxonomic group, treating them as separate inputs to the cumulative invasive species threat dataset. For plants, individual ensemble models were reclassified to 0,1 based on a 70% model threshold and then summed together to create invasive plant species richness layer. We generated minimum convex hulls around occurrence data for individual invasive reptile species as a proxy for the species' invasive range. Given the widespread distribution of non-indigenous aquatic species and specificity to freshwater systems, we did not use convex hulls to determine range of these species. Instead, we used the actual occurrence points rasterized to 1km to determine range of each species. Range map data were rasterized to 1km resolution.

Overexploitation: We used datasets on recreational freshwater fishing and total harvestable species richness (EPA EnviroAtlas; Pickard *et al.* 2015). Big game recreational demand and migratory bird recreational demand were highly spatially correlated with freshwater fishing recreational demand and therefore were removed from the analysis. These datasets are summarized at a HUC-12 spatial scale. Recreational demand was estimated from USFWS Fishing, Hunting, and Wildlife-Association Recreation surveys to summarize annual day trips for each watershed. Total harvestable species richness refers to the number of species that may be hunted or trapped, including big and small game, waterfowl, and fur-bearer species. Species richness was calculated from habitat models, and total number refers to the highest species richness recorded for each watershed.

Pollution: We considered nine variables that were uncorrelated: Total annual sulfur deposition (kg/ha), Stream length impaired by pH, acidity, or caustic conditions (km), Stream length impaired by organic enrichment or oxygen depletion (km), Stream length impaired by nutrients (km), Stream length impaired by metals other than mercury (km), Stream length impaired by mercury (km), Surface runoff from agricultural land (mm) (EPA EnviroAtlas). These datasets are at a HUC-12 spatial scale.

DRIVER	METRIC	SOURCE
CLIMATE CHANGE	Climate Dissimilarity based on 11 biologically-relevant temperature and precipitation variables, RCP 4.5, 2080s.	Belote et al, 2018
	Climate velocity based on A2 emissions scenarios implemented by seven GCMs of the CMIP3 multimodel dataset, RCP 4.5, 2080s.	AdaptWest Project 2015
INVASIVE SPECIES	Global Avian Invasions Atlas -bird species range - shapefiles	Dyer et al, 2017
	Distribution of Alien Mammals – mammal species range - shapefiles	Biancoline et al, 2021
	Reptiles – point occurrence	Wiens et al, 2019
	Non-indigenous aquatic species – point occurrence	NAS.USGS
	INHABIT database – invasive plant species distribution models	Jarnevich et al. 2023
LAND USE CHANGE	Predicted land-use change 2005-2075 using pathway scenario B1	USGS LULC, Sohl et al, 2014
OVER-EXPLOITATION	Freshwater fishing recreation demand (day trips per year), Total harvestable species richness – Maximum	EPA-enviroAtlas
POLLUTION	Total annual sulfur deposition (kg/ha), Stream length impaired by pH, acidity, or caustic conditions (km), Stream length impaired by organic enrichment or oxygen depletion (km), Stream length impaired by nutrients (km), Stream length impaired by metals other than mercury (km), Stream length impaired by mercury (km), Surface runoff from agricultural land (mm)	EPA-enviroAtlas

Threat Hotspots

Hotspots for each threat dataset were identified based on the 90th percentile: top 10% of the contiguous U.S. Hotspots for each separate threat were combined to determine areas of cumulative threat exposure.

Ecoregional deviations in threat values among imperiled biodiversity-rich areas were analyzed to understand variability in threat exposure across ecoregions in the contiguous U.S. (Figure S2; EPA level III ecoregions; EPA 2010). For this analysis, we only considered the threat values associated with areas of biodiversity importance for each ecoregion (90th percentile) to avoid zero-inflation from included non-biodiversity hotspot locations. We first measured overall mean for each driver across all ecoregions and then measured the deviation between all grid cells of each region to that overall mean so that values higher than 0 represented grid cells with values higher than the overall mean. Finally, we measured the mean deviation within each ecoregion.

Areas of Biodiversity Importance

Areas of biodiversity importance were based on range-size rarity data from the Map of Biodiversity Importance project (NatureServe Network 2021; Hamilton *et al.* 2022). This dataset is the summed range-size rarity of species in the lower 48 United States that are protected by the Endangered Species Act and/or considered to be in danger of extinction (NatureServe category G1 or G2). The dataset is based on habitat suitability models for 2,216 of the nation's most imperiled vertebrate, vascular plants, freshwater invertebrate and pollinator species. High values identify areas where species with very small ranges (and thus fewer places where they can be conserved) are likely to occur; the presence of multiple imperiled species contributes to higher scores. Areas of highest biodiversity importance were identified based on the 90th percentile: top 10% of the contiguous U.S. Only these locations were used in analyses.

Because potential impacts to specific species could not be distinguished from the pooled biodiversity data, we also analyzed individual species ranges for those listed as threatened or endangered under the Endangered

Species Act that are found in the continental U.S. (freshwater or terrestrial species or marine species with terrestrial habitat). Ranges for 958 listed species were gathered from U.S. Fish and Wildlife Service's Environmental Conservation Online System.

Overlaps (Important Biodiversity Areas at Higher Risk of Exposure)

We measured extent of overlap between threat hotspots (separate and cumulative) and areas of biodiversity importance for the four taxonomic groups (vertebrate, vascular plants, freshwater invertebrate, and pollinators) and combined (Figures 2, 4 & S3, Table S1). To assess spatial relationships between cumulative threat intensity and biodiversity importance, we measured terciles (i.e., 33% and 66% percentiles) for cumulative threat ranking and imperiled range-size rarity, in order to categorize pixels with low (pixels with values under 33% percentile), medium (values between 33% and 66% percentiles) and high threat ranking or biodiversity importance (values higher than 66%). We used this matrix to identify important biodiversity areas at risk of exposure to the five threats.

We quantified imperiled species range overlaps with the threats that were identified as contributing to their endangerment (Table S2; see *Species' Threats Determination*). Data were summarized by threat, taxonomic group and for small-range species. Small-range species were defined as those whose range sizes fell in the first quartile. T-tests were conducted to determine significant differences in the mean proportion of range overlapping each threat hotspot and multiple hotspots between small-range species and others.

Data Limitations

Spatial

There are some inherent limitations to the data used in this analysis. For example, in absence of spatial data explicitly developed to measure the extent of a particular threat, we used proxies (e.g., exploitation). For others, we only focused on a small subset of the threat: spatial data for soil and water pollutants and not light, air, or others. Additionally, spatial datasets on species diversity will always be biased toward the taxonomic groups for which spatial data is available and only give a partial picture of biodiversity. Species-specific overlaps with threat hotspots were based on range data, which provide valuable perspective on patterns at larger scales, but are less useful for identifying areas for local conservation action as they also include areas of unsuitable habitat. Additionally, species with larger ranges (i.e., birds, bats, non-volant migratory mammals, etc.) are less likely to have higher proportion of overlap with threat hotspots. Additional threat metrics should be considered to account for this. Most data represent the current state of threats and habitat suitability, much of which may shift with global climate change. Future local, regional, and continental scale analyses can help inform which areas need long-term protections.

Focusing on values at the national scale means that entire ecosystems important to representing local species assemblages and key ecosystem services are not included on the map. While we took a stratified approach for assessing ecoregional deviations in threat exposure, other parts of our analysis can be modified to ensure that threats are assessed for all native ecosystems and their associated areas of biodiversity importance. We did not account for current land designations, management practices, or traditional ecological knowledge, all of which will be important to consider for assessments done a smaller-scales.

Aspatial

To assess threats to species we took a conservative approach and used an amalgamation of threats listed from multiple different platforms. It is possible that some of these threats have changed since time of listing (e.g. if a species was listed on the ESA, overharvesting of that species may have decreased). While this data provides important insight into trends for species at large as well as species groups, management plans for specific species should reassess threats periodically to ensure that management is focused on the most salient issues for a given species. We also only included species that have been listed under the ESA or IUCN Redlist in our analyses. The ESA does not include all imperiled species and the IUCN Redlist is an ongoing project to assess species. Invertebrates, among other groups, are likely disproportionally unaddressed.

Finally, we acknowledge the strong need for several additional considerations not accounted for in this work. In addition to the primary focus on biodiversity, developing a National Nature Assessment relevant to robust conservation policy and action will require addressing issues related to economic, political, and social constraints. Future work should also help to more explicitly identify opportunities for improving human health, well-being, and equitable access to nature. Goals to ensure a healthy environment for all communities have long been ignored or discounted in protected areas designations, in part because these topics are not well studied. Last, it is worth noting that variation in the ways that people value biodiversity and habitats varies, something that the leaders of the National Nature Assessment have sought to account for in their solicitation for public comment.

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Appendix I: Additional Figures and Tables

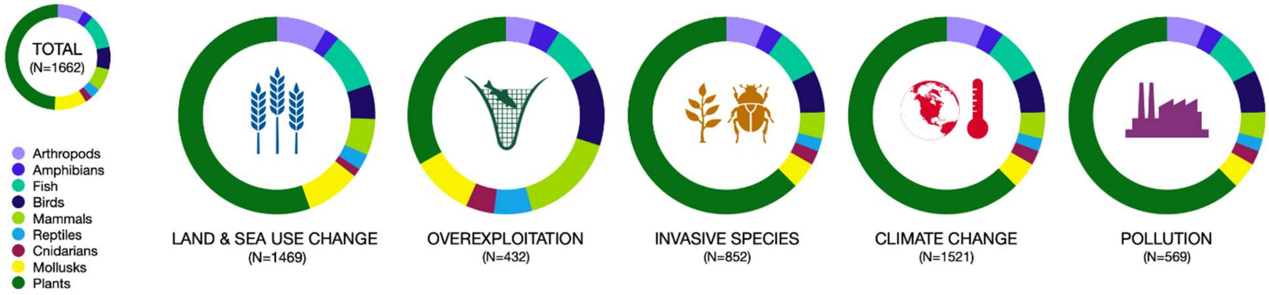

Figure S1: Taxonomic composition of species threatened by each of the five main drivers of global biodiversity loss.

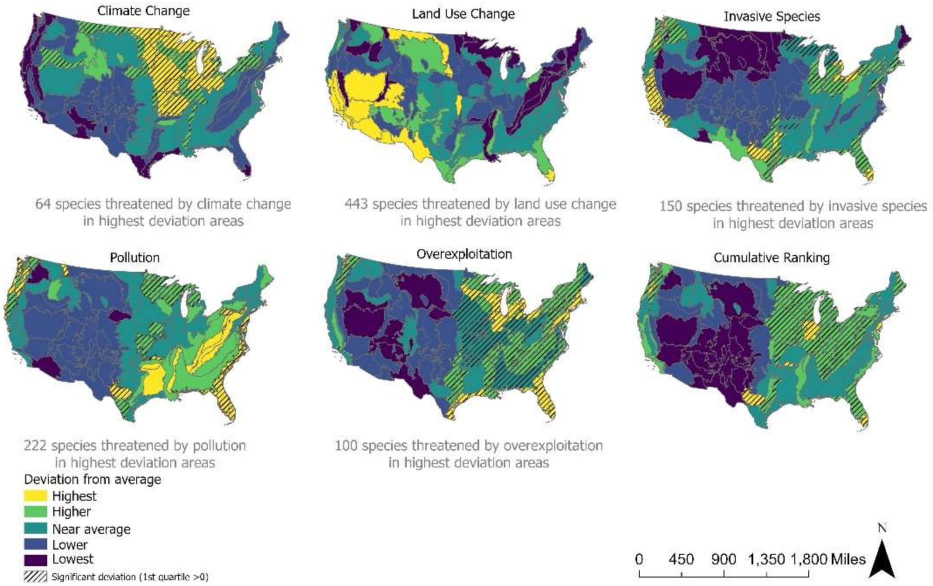

Figure S2: Ecoregional deviations from the overall mean (continental US) of each threat by ecoregion level 3. Values above the mean represent regional values higher than overall mean and values below the mean represent regional values lower than overall mean. Hatched regions show distributions where 1st quartile is higher than zero, meaning distribution of values in ecoregions is significantly higher than overall mean.

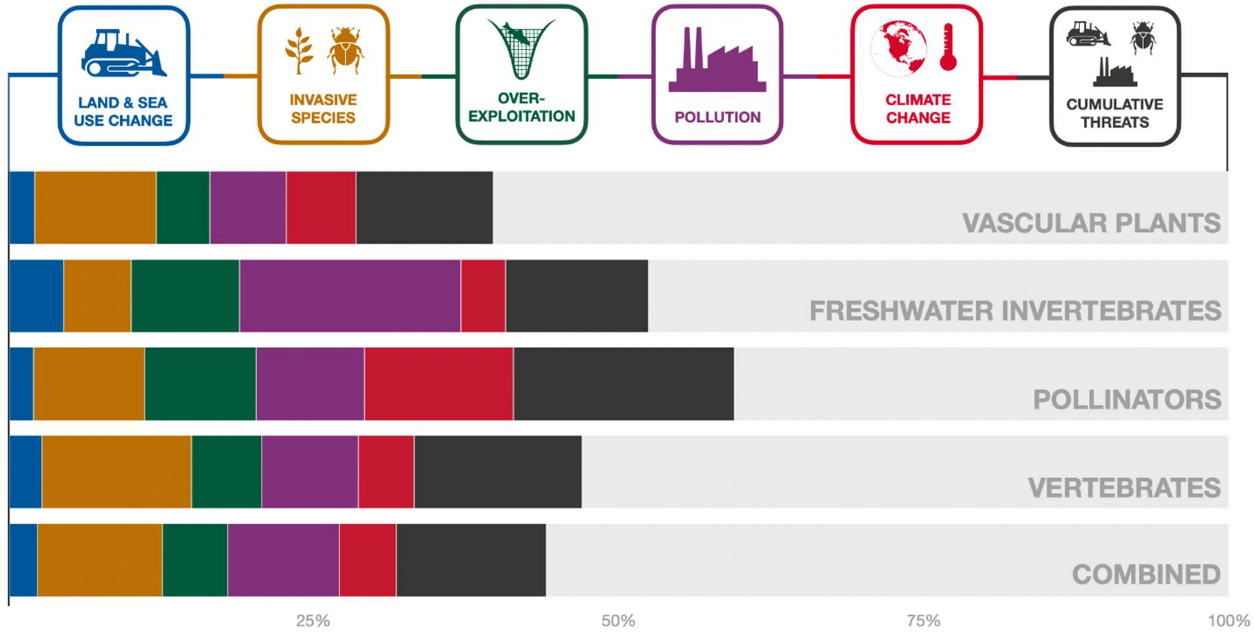

Figure S3: Areas with the highest values of biodiversity importance (top 10%, NatureServe's Map of Biodiversity Importance) for imperiled vascular plants, freshwater invertebrates, pollinators, vertebrates and all species combined were overlayed with areas of high exposure (top 10% of the contiguous U.S. for each threat) for each of the five underlying drivers of biodiversity loss: land- and sea-use change, invasive species, overexploitation, pollution and climate change. Bars indicate the percent of overlap. Colors and symbols correspond to the five main threats to global biodiversity loss, with black indicating more than one threat. Some of the most ubiquitous threats (e.g., land and sea use change and climate change may appear as smaller slices of the bar chart because of their overlap with other threats).

Table S1: Overlap between imperiled species hotspots and threat hotspots.

Taxonomic Group(s)	Pollution (%)	Overexploitation (%)	Invasive Species (%)	Land-Use Change (%)	Climate Change (%)	2+ (%)
Plant	6.26	4.39	9.97	2.14	5.70	11.26
Freshwater	18.14	8.89	5.51	4.52	3.63	11.74
Pollinators	8.87	9.10	9.11	2.05	12.21	18.09
Vertebrates	7.91	5.76	12.25	2.74	4.54	13.75
All	9.14	5.36	10.26	2.36	4.65	12.28

Table S2: Listed species with at least 90% of their range overlapping threat hotspots. For listing under the Endangered Species Act (ESA), T = Threatened and E = Endangered. Under threat columns, 1 indicates that the threat is contributing to species' endangerment. *For the California tiger salamander, both endangered distinct population segments are included.

Scientific Name	**Common Name**	**Taxon**	**ESA Status**	**Climate Change**	**Land-Use Change**	**Invasive Species**	**Overexploit**	**Pollution**	**Prop Range Threat**
Ambystoma californiense	California tiger salamander	Amphibian	E*	1	1	1	0	1	1.00
Thomomys mazama pugetensis	Olympia pocket gopher	Mammal	T	1	1	1	1	0	1.00
Penstemon penlandii	Penland beardtongue	Plant	E	1	1	0	1	0	1.00
Rhinichthys osculus thermalis	Kendall Warm Springs dace	Fish	E	1	1	1	0	0	1.00
Lirceus usdagalun	Lee County cave isopod	Invertebrate	E	1	0	0	0	1	1.00
Gila bicolor	Hutton tui chub	Fish	T	1	1	0	0	1	1.00
Eriogonum ovalifolium var. williamsiae	Steamboat buckwheat	Plant	E	1	1	0	0	1	1.00
Ambrysus amargosus	Ash Meadows naucorid	Invertebrate	T	1	1	0	1	0	1.00
Stephanomeria malheurensis	Malheur wire-lettuce	Plant	E	1	0	1	0	0	1.00
Thomomys mazama tumuli	Tenino pocket gopher	Mammal	T	1	1	1	1	0	1.00
Euphydryas anicia cloudcrofti	Sacramento mountains checkerspot butterfly	Invertebrate	E	1	1	1	1	0	1.00
Cirsium loncholepis	La Graciosa thistle	Plant	E	1	1	1	0	0	1.00
Dudleya stolonifera	Laguna Beach liveforever	Plant	T	1	1	1	1	0	1.00
Asimina tetramera	Four-petal pawpaw	Plant	E	1	1	1	1	1	1.00
Thomomys mazama glacialis	Roy Prairie pocket gopher	Mammal	T	1	1	1	1	0	1.00
Etheostoma nianguae	Niangua darter	Fish	T	1	1	0	0	1	1.00
Anaea troglodyta floridalis	Florida leafwing butterfly	Invertebrate	E	1	1	1	1	1	0.99
Cyprinodon nevadensis pectoralis	Warm Springs pupfish	Fish	E	1	1	1	0	0	0.99
Thomomys mazama yelmensis	Yelm pocket gopher	Mammal	T	1	1	1	1	0	0.99
Chasmistes liorus	June sucker	Fish	T	1	1	1	1	1	0.98
Sylvilagus bachmani riparius	Riparian bush rabbit	Mammal	E	1	1	1	1	0	0.98
Rorippa gambellii	Gambel's watercress	Plant	E	1	1	1	1	0	0.98

Consolea corallicola	Florida semaphore cactus	Plant	E	1	1	1	1	1	0.98
Fremontodendron mexicanum	Mexican flannelbush	Plant	E	1	1	1	0	0	0.98
Cambarus aculabrum	Benton County cave crayfish	Invertebrate	E	1	1	0	1	1	0.96
Dipodomys stephensi	Stephen's kangaroo rat	Mammal	T	1	1	1	1	1	0.96
Galium californicum ssp. sierrae	El Dorado bedstraw	Plant	E	1	1	1	0	1	0.95
Cicindela ohlone	Ohlone tiger beetle	Invertebrate	E	1	1	1	1	0	0.95
Lanius ludovicianus mearnsi	San Clemente loggerhead shrike	Bird	E	1	1	1	1	1	0.95
Astragalus osterhoutii	Osterhout milkvetch	Plant	E	1	1	0	1	0	0.94
Lampsilis strecheri	Speckled pocketbook	Invertebrate	E	1	1	0	1	1	0.93
Speyeria callippe callippe	Callippe silverspot butterfly	Invertebrate	E	1	1	1	1	0	0.93
Etheostoma moorei	Yellowcheek darter	Fish	E	1	1	0	0	1	0.93
Amblyopsis rosae	Ozark cavefish	Fish	T	1	1	0	1	1	0.93
Nitrophila mohavensis	Amargosa niterwort	Plant	E	1	1	0	0	0	0.92
Anaxyrus californicus	Arroyo toad	Amphibian	E	1	1	1	1	1	0.92
Menidia extensa	Waccamaw silverside	Fish	T	1	0	0	0	1	0.90
Cottus paulus	Pygmy sculpin	Fish	T	1	1	0	0	1	0.90

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